Synopsis of Age Validation Study of Gray Triggerfish through Chemical Marking

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SEDAR82-DW03

Received: 7/25/2022

Please cite this document as:

Potts, Jennifer C., et.al. 2022. Synopsis of Age Validation Study of Gray Triggerfish through Chemical Marking. SEDAR82-DW03. SEDAR, North Charleston, SC. 22 pp.

Synopsis of Age Validation Study of Gray Triggerfish through Chemical Marking

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July 25, 2022

Introduction

The age and growth of Gray Triggerfish, *Balistes capriscus*, have been investigated throughout the species range including the Mediterranean Sea, eastern Atlantic Ocean along the coast of Central Africa, off Brazil, and the western Atlantic Ocean along the U.S. South Atlantic and Gulf of Mexico (Table 1 – peer-reviewed articles). The life history of Gray Triggerfish has been the subject of several student theses and dissertations, reports and peer-reviewed articles. The primary age structure used in these studies has been the first dorsal spine. The spine is easily excised from the fish and shows alternating slow and fast growth layers presumed to be annuli. Another reason the spine has been preferred for ageing is because the sagittal otoliths are very small, fragile and difficult to extract (Bernardes, 2002; Milazzo et al. 2004; Allman et al. 2016), especially from the perspective of the port agent on the docks or in fish houses who collect age samples for production ageing laboratories (Burton et al., 2015). Also, sagittal otoliths have a unique shape, causing difficulty when determining the best spatial plane upon which to age and/or section the structures (Shervette et al. 2020). More research on the otoliths may resolve some of the processing issues.

All of the ageing studies on this species have noted the difficulty in interpreting the growth zones on spines (Bernardes, 2002; Kelly-Stormer et al. 2017). The lack of marginal increment validation of the periodicity of the growth zones has left age readings in question. Bernardes (2002) and Allman et al. (2016) reported that two translucent zones could be formed each year, while other studies reported just one translucent zone per year. Jefferson et al. (2018), Ingram (2001), and Caverieri et al. (1981) discussed the formation of the first annulus, but did not agree specifically on which translucent zone was the first annulus or how close to

the focus it was formed. Because of these issues, validation of age readings from spines of Gray Triggerfish was needed.

There have been attempts to validate growth zone deposition on the spines of Gray Triggerfish. In an unpublished Marine Fisheries Initiative (MARFIN) study funded by NOAA National Marine Fisheries Service, Hood and Johnson (1997) utilized two age validation methods: marginal increment analysis of dorsal spines and captive rearing of oxytetracycline (OTC)- marked fish. The marginal increment analysis did not show a clear pattern of annulus formation, and the amount of growth zone formation following the OTC mark on the spine sections from the chemically marked fish was inconclusive. Another validation study was conducted by Allman et al. (2016). They captured eight fish from offshore habitats, marked them with OTC, and held them in an aquaculture facility with ambient light and mean seasonal bottom temperatures from the capture area. Four of the fish survived for a period of 262 days (October to July). Allman et al. (2016) examined dorsal spines, fin rays, and vertebrae sections from each of those fish, and all structures showed one annulus (translucent zone) forming in the late winter months. Though the results of the OTC experiment indicated one annulus formed per year, the marginal condition analysis on an additional 2,411 spine samples indicated that a second translucent zone appeared to form in the fall of the year (September). Further study on growth zone formation on age structures of Gray Triggerfish was warranted.

The primary objective of this study was to validate the annual deposition of growth zones on the first dorsal spines, vertebrae, and otoliths of Gray Triggerfish by capturing fish from surface and bottom habitats, chemically marking them, and holding them in an aquaculture facility for greater than two years. The resulting age readings and interpretation of

the macro-structure from the various structures were compared. The results of this study will be applied to production ageing statistics and have implications for use in stock assessments.

Methods

Gray Triggerfish were collected off the coast of North Carolina via hook and line (n=69) during December 2014 and spring 2016, and opportunistically collected from traps (n=19) and surface dip net (n=13) in summer of 2014 through Spring 2016 during various research cruises. Live fish were transported to NOAA Beaufort Laboratory marine aquaculture facilities, and reared in recirculating aquaculture systems (RAS) equipped with mechanical filtration and bio-filtration along with an UV sterilizer for disinfection. The adult fish were held in two RAS systems, each consisting of three 2.3 m^3 (600 gallon) round fiberglass tanks. The juvenile fish were reared in a RAS system consisting of three 0.5 m^3 (130 gallon) semi-round polyethylene tanks. These systems were housed in a climate-controlled facility and had inline heat pumps and chillers to adjust water temperature to reflect offshore bottom temperature data collected from long-term monitoring programs conducted at the NOAA Beaufort laboratory (Figure 1). Skylights were installed in the aquaculture facility to allow ambient light into the rearing tanks, ensuring natural diurnal light cycles. Gray Triggerfish were fed a daily diet of cut squid and commercial marine finfish pellets, and uneaten food was removed from the bottom of tanks after feeding.

Adult Gray Triggerfish were chemically marked on March 25, 2015 (n = 47), March 1, 2016 (n = 2), June 16, 2016 (n = 12), and October 11, 2016 (n = 13). Live fish were anesthetized by submersion in a 75 mg/L solution of Tricaine-S and intramuscularly injected with a 50 mg/kg body weight dose of calcein (Monaghan, 1993). To make the injectable solution, calcein was

mixed into a 0.9% bacteriostatic solution buffered to a pH of 7.3 at a concentration of 60mg/mL. Following injection, fish were returned to rearing tanks and allowed to recover.

Spines, vertebrae, and otoliths were removed from the experimental fish and subsequently processed to obtain age estimates. All three ageing structures were thin sectioned for analyses, though the sagittal otoliths were viewed whole and imaged prior to sectioning. Each prepared structure was aged individually by two readers without reference to the other structures, size of the fish, date of death or date of marking. Spines were read following the protocol set during an age workshop held in 2013 with experts from around the U.S. Southeast region and used in Burton et al. (2015) and for SEDAR 41 data (SEDAR, 2016). The sections from each of the age structures were read primarily under reflected light using a stereomicroscope at 15-40x magnification, but were also viewed using transmitted light for comparison. Narrow, slow-growth zones appeared translucent in spine and vertebra sections, and opaque in otolith sections. These slow-growth zones were counted to estimate the age of the fish. Consensus ages were determined for any conflicting reader estimates. Paired comparisons of annuli counts were conducted among the three ageing structures, and bias plots were created to visualize age differences attributed to each structure. Based on the recommendation by McBride (2015), Evans and Hoenig (1998) and Bowker (1948) tests for symmetry were performed, followed by a Breusch-Pagan tests for heteroscedasticity.

Further analyses of the data collected in this study focused on the age estimates from spines compared to otoliths. Spines have been the primary age structure for Gray Triggerfish, but other research suggests that otoliths are more reliable for ageing fish in general. Von Bertalanffy growth models from the age data from each structure, overall and paired data,

were estimated and then compared to the population growth model recorded in SEDAR 41 (SEDAR, 2014).

Results

A total of 101 Gray Triggerfish were successfully held in tanks during the study, and 74 were marked with calcein. Fish injected with calcein survived from 5 days to 29 months after marking (Figure 2). Those marked in March 2015 (n=47) were held for an average of 24 months, while fish marked in March 2016 (n=2) were held for 16 months; marked in June 2016 were held for 1.5 months (n = 12; water quality issue caused all fish in tank to die); and marked in October 2016 were held for an average of 9 months (n = 13). The remaining fish (n = 27) not marked with calcein were some of the smallest fish in the study and were captured in traps or with dipnets at opportunistic times.

Annuli counts were enumerated for spines (n=96), vertebrae (n=94), and otoliths (n=48) and ranged from 0-11 for spines and vertebrae, and 1-12 for otoliths (Figure 3). Fish ranged in size from 31 – 498 mm fork length (FL; Figure 4). Some check marks, or doublets, were noted on the spines and vertebrae, but not on the otoliths (Figure 5). These check marks were discontinuous around the entire spine structure, while the presumed true annulus was continuous. On the vertebrae, the annuli could be seen crossing the entire section, but the check marks were discontinuous and very closely spaced with the presumed true annulus.

Comparisons of age readings between the pairs of age structures were analyzed with different methods. Age bias plots showed strong agreement between spine and vertebra annuli counts for all observed ages, and counts of spines and vertebrae appeared to underage

beginning at age 7 when compared to otolith annuli counts (Figure 6). Further comparisons will focus on spine and otolith age data. Spine ages agreed with otolith ages 52.2% of the time and 82.6% within ±1 year (APE = 8.8% and CV = 12.4%). The Evans and Hoenig (1998) and Bowker (1948) tests indicated that the paired age data were not biased (p >0.05). The test of heteroscedasticity was highly significant for paired spine and otolith data (p <<0.05), but was not significant for paired spine and vertebrae data (p > 0.05). These results were expected after examination of the bias plots.

Translucent and opaque growth zones appeared to form in all ageing structures during the captive rearing period, and calcein marks were observed in spine (n=68), vertebrae (n=72), and otolith (n=37) sections (Figure 7). The expected number of post-mark annuli was present in 62 of 68 spine sections, 65 of 72 vertebrae sections, and in all of the otolith sections (Figure 8). The six spine samples where the expected growth after the mark was not seen were on fish ages 3, 4, 5 (2x), 6, and 10. Of these, the difference in the paired otolith reading (n = 4) was ±1 year. The seven vertebrae samples where the expected growth was not seen were on fish ages 3, 4, 5, 6, 7 (2x), and 11. Of these, the difference in the paired otolith reading (n = 3) was ±1 year.

The von Bertalanffy growth models were generated based on the spine and otolith calendar age for all data available for each structure and for the data where the readings were paired. The resulting parameter values are presented in Table 2 (Figure 9).

Conclusions

Some results of this study lend confidence to the ageing of Gray Triggerfish with spines. The range of lengths and ages of the fish in our study was similar to those found in the population in the U.S. South Atlantic (SEDAR, 2016). The maximum age of the fish in our study was 12 years, which was estimated from an otolith. The maximum age from the spine sections was 11 years, but unfortunately we did not have the otolith from the same fish for comparison. In SEDAR 41 (SEDAR, 2016), the maximum age from spine readings was 15 years. Given the small sample size in our study, the fact that we had a fish to age 12 might suggest that the fish may live longer than 15 years, but we cannot be sure.

The validation of the annual deposition of growth zones on spines, vertebrae and otoliths was generally achieved. The expected number of growth zones, or annuli, after the chemical mark on the otoliths was present. Greater than 90% of the spines also had the expected number of growth zones present. The ages estimated from the paired otoliths and spines showed good agreement to age-7. In the SEDAR 41 age data set, 88% of the fish were ≤ 5 years old, which suggests that using age data from reading spines would give the assessment model the majority of the population.

One concern about potential under-estimate of ages is the effect on the growth model. If the ages are under-estimated, the growth of the fish will appear to be faster than what is happening in reality. The age data from the spines did cause the growth model to have a higher L_∞ than what was estimated from the age data from otoliths. Because the K estimated from the otolith ages was higher, the estimates of yield from the fishery may be influenced. On the other hand, both growth models show that the majority of the L_∞ is achieved by age-5 – 88%

from spine data and 98% from otolith data. The SEDAR 41 population growth model fell in between our spine and otolith data models. Because the data from this study were limited by sample size, the growth model information should be used cautiously.

All analyses presented in this report were based on the consensus age readings, but upon closer inspection, some of the spine sections exhibited unusually wide translucent zones as seen at lower magnification (15x - 20x). We re-examined those spine sections under higher magnification (up to 40x). The wide translucent areas were actually compacted growth layers (Figure 10). Once those growth layers were counted, the annuli count on the spines, matched the age from the otolith, especially in the oldest fish. Our study suggests changing the age reading methodology as used in SEDAR 41 to include the closer examination of the spine sections for compacted growth layers.

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Table 1. Published life history studies of Gray Triggerfish *Balistes capriscus* with information specifically about age and growth characteristics

Study	Location	Age Structure	Validation method	Max age (yrs) reported
Johnson and Saloman, 1984	U.S. Gulf of Mexico – NW Florida	First dorsal spine	Monthly frequency of annulus (translucent zone) on margin	13
Burton et al., 2015	U.S. Southeastern Atlantic	First dorsal spine	Marginal Increment Analysis	15
Kelly-Stormer et al., 2017	U.S. Southeastern Atlantic	First dorsal spine	Monthly frequency of annulus (translucent zone) on margin	12
Jefferson et al., 2019	U.S. Gulf of Mexico – Alabama	First dorsal spine	Monthly frequency of annulus (translucent zone) on margin	10
Shervette et al., 2020	U.S. South Atlantic – North Carolina and South Carolina	First dorsal spine and whole sagittal otoliths	None reported	Spines (sp)= 11; Otoliths (ot) = 13
Shervette et al., 2020	West Africa - Ghana	First dorsal spine	None reported	9
Caveriviere et al., 1981	West Africa – Senegal and Ivory Coast	First dorsal spine		Senegal (S) = 6 Ivory Coast (IC) = 7
Ofori-Danson, 1989	West Africa - Ghana	First dorsal spine	None reported	4
Aggrey-Fynn, 2001	West Africa – Western Gulf of Guinea	First dorsal spine	None reported	11
Bernardes, 2002	Brazil	First dorsal spine	Monthly frequency of annulus (translucent zone) on margin	11
İşmen et al., 2004	Mediterranean Sea İskenderun Bau	First dorsal spine	None reported	3
Milazzo et al., 2004	Mediterranean Sea – Strait of Sicily	First dorsal spine and whole otolith	None reported	7
Kacem et al., 2015	Mediterranean Sea – Gulf of Gabès	First dorsal spine	Marginal increment analysis	13

Table 2. Von Bertalanffy Growth parameters estimated from spine and otolith age data from age validation study. All available data were used for separate spine and otolith models; all available pairs of matching spines and otoliths were included in the pair comparison models. SEDAR 41 population growth model parameters are included for reference (SEDAR, 2016)

	Age Structure	**L_∞**	**K**	**t_0**
All data available	Spine (n = 96)	485.5	0.33	-0.61
	Otolith (n = 48)	432.3	0.60	-0.08
Paired spine and otolith readings	Spine (n = 46)	476.7	0.35	-0.74
	Otolith (n = 46)	431.5	0.60	-0.09
SEDAR 41	N = 8,102	453.2	0.34	-0.98

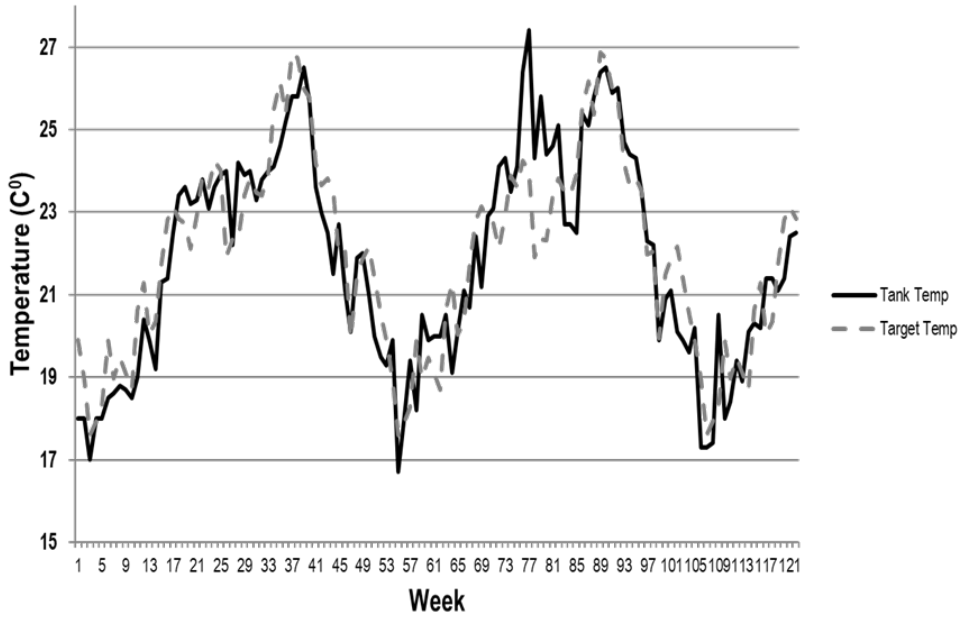


Figure 1. Water temperature recorded by data loggers deployed on Gray Triggerfish habitat off of North Carolina overlaid with the water temperatures maintained in the holding tanks for the fish in the age validation study.

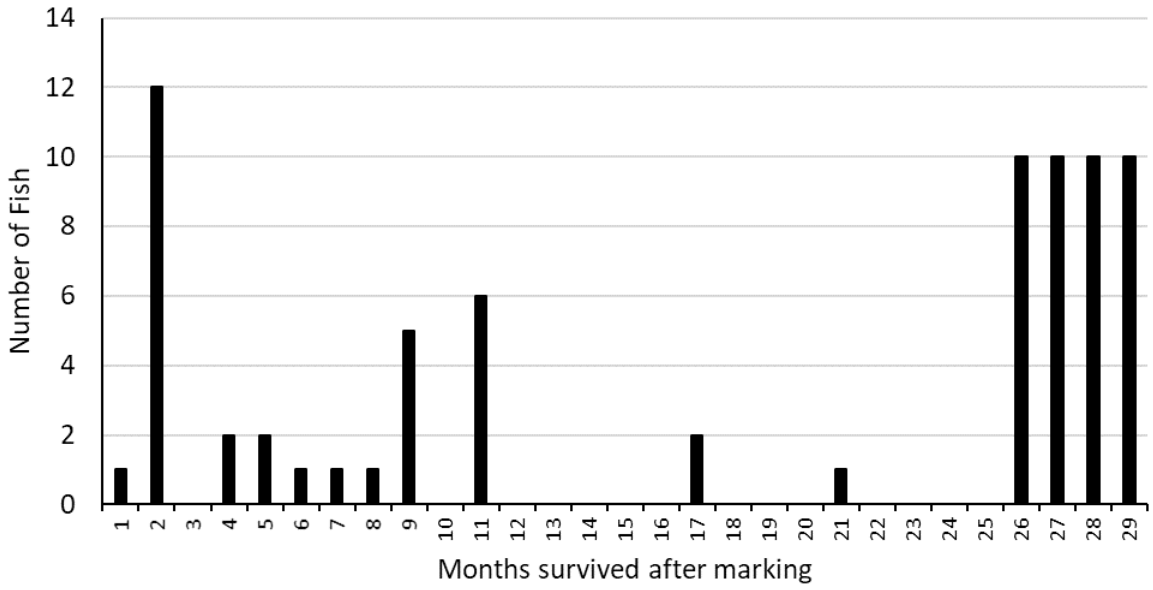


Figure 2. Survival time after chemical marking of Gray Triggerfish in the age validation study.

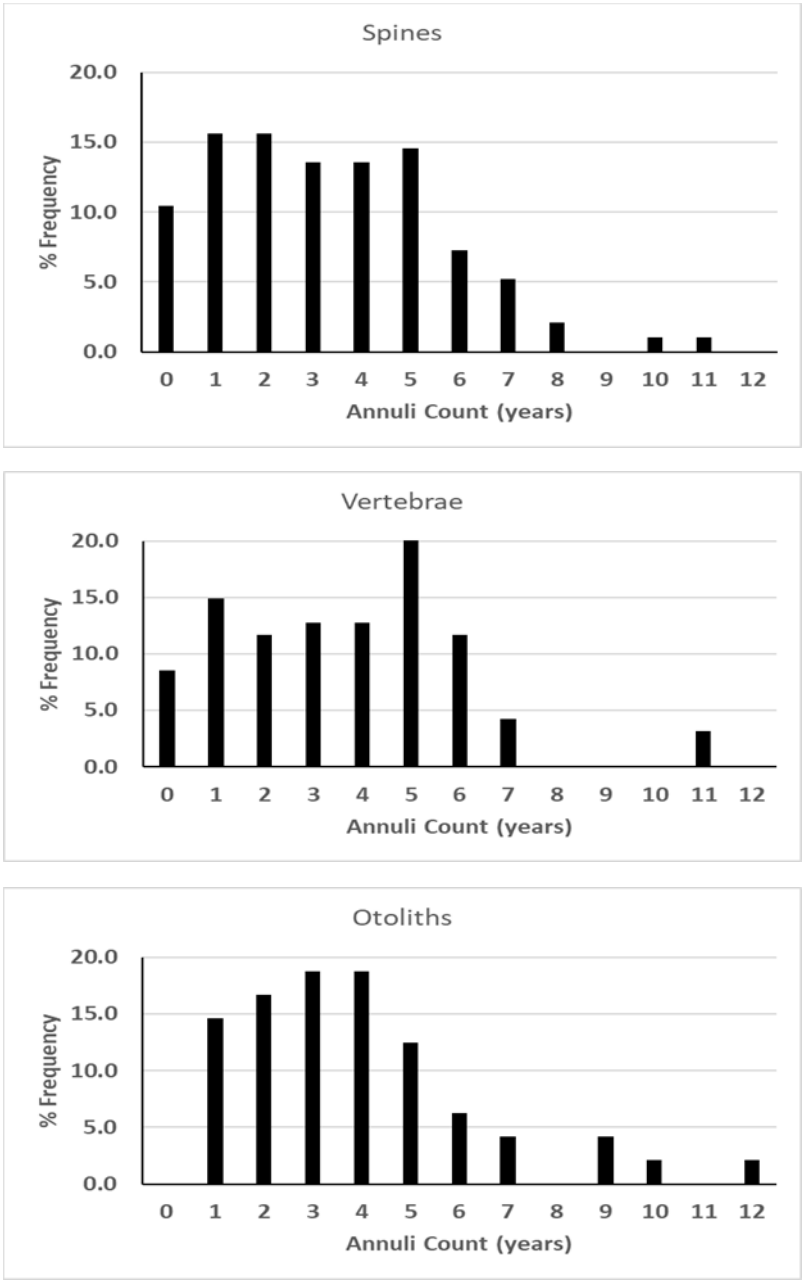


Figure 3. The age frequency of Gray Triggerfish from the age validation study by age structure: Spines (n = 96), vertebrae (n = 94), and otoliths (n = 48).

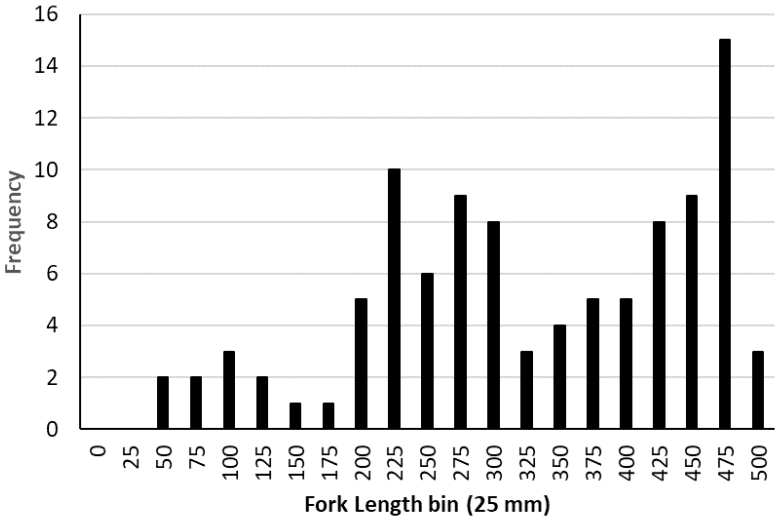


Figure 4. Length frequency of the Gray Triggerfish in the age validation study.

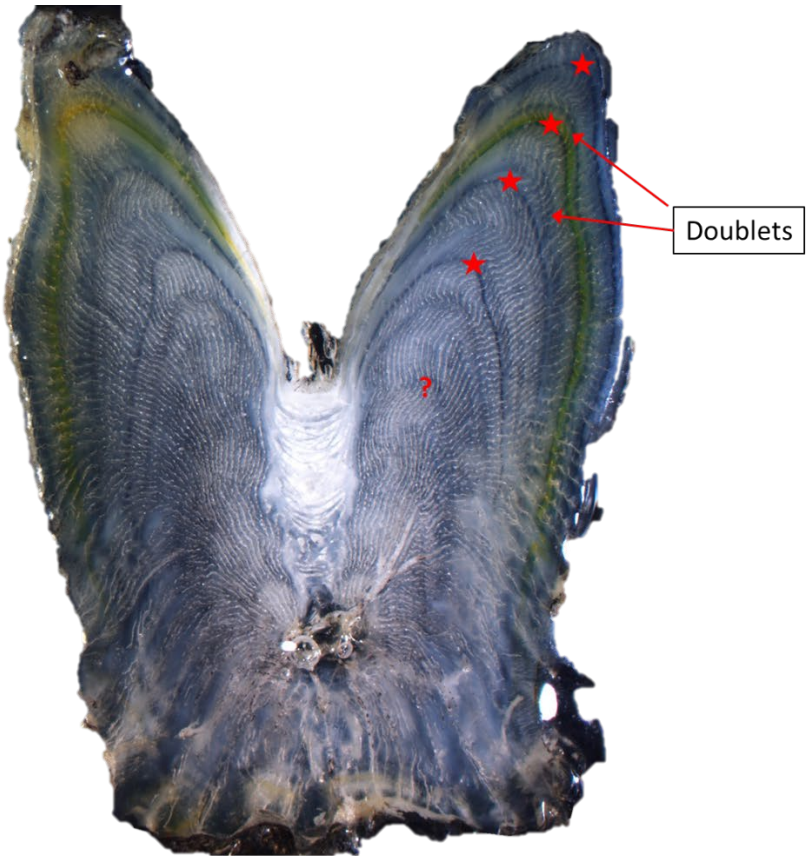
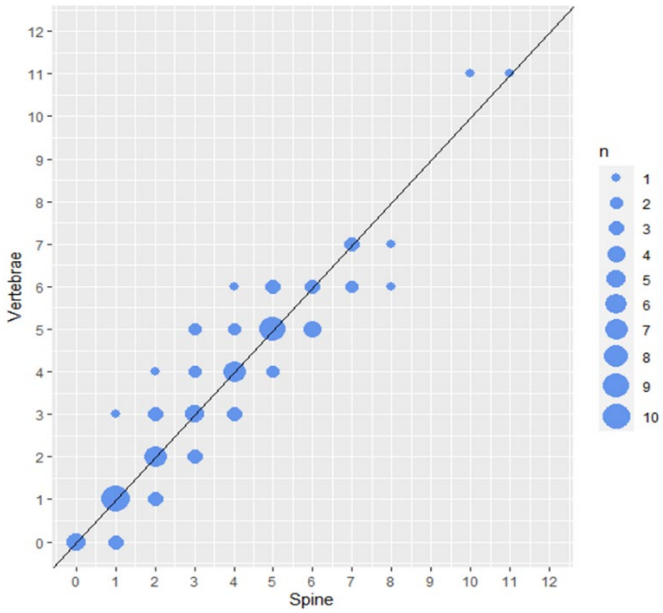


Figure 5. Gray Triggerfish spine section exhibiting doublets (aka check marks). Red stars indicate annuli and the "?" is another possible annulus. Yellow band on spine is the calcein mark that was made in March 2015. The fish was sacrificed in June 2017. The growth after the mark was 2 years and the 2017 annulus had not formed yet.

A.



B.

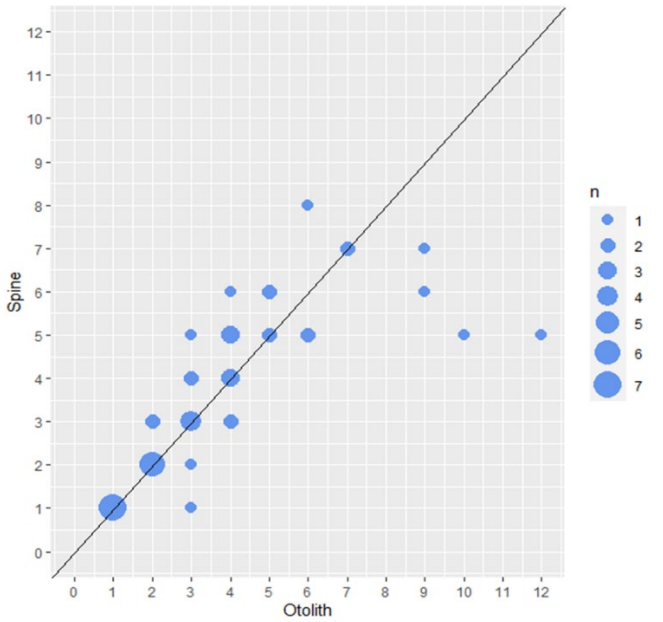


Figure 6. Age bias plots of Gray Triggerfish age structures in the age validation study. Plot A is the vertebrae ages compared to the spine ages. Plot B is the spine ages compared to the otolith ages.

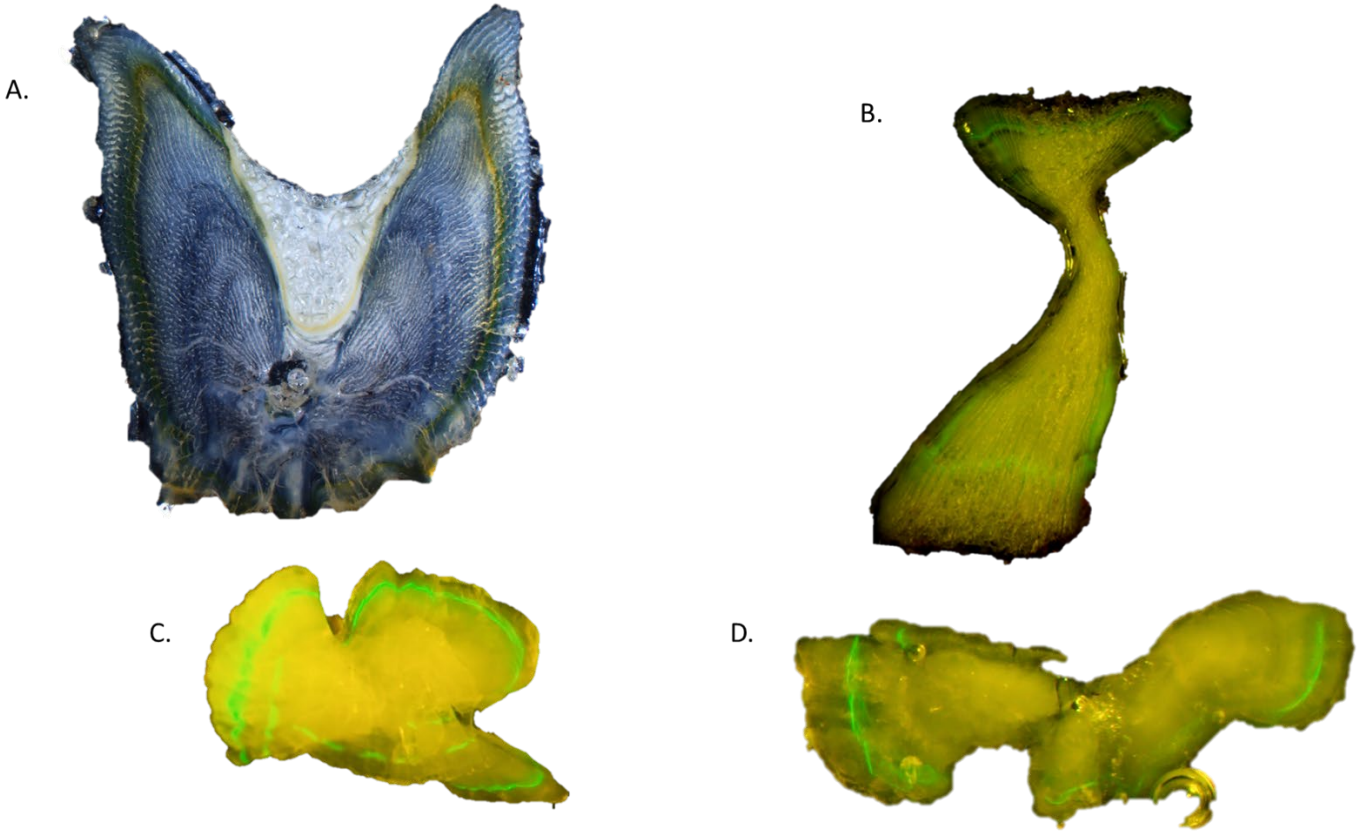
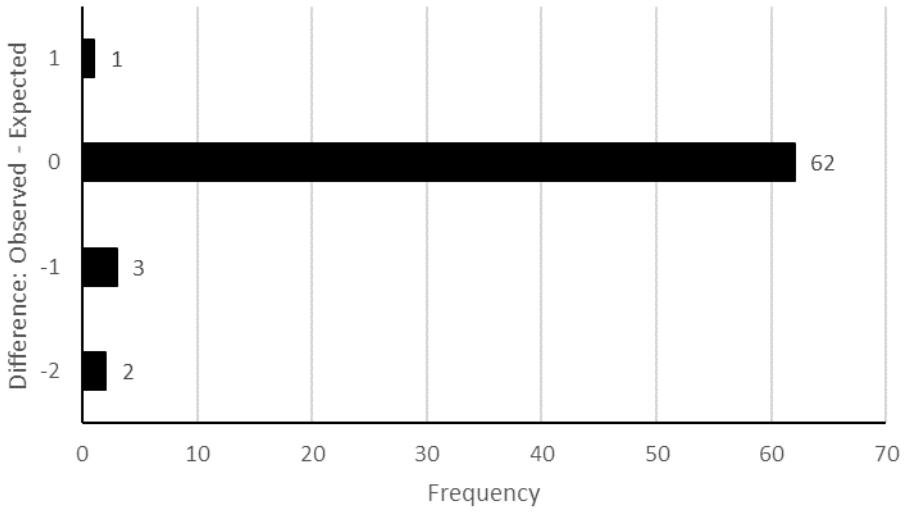


Figure 7. Spine section (A), vertebra section (B), whole sagittal otolith (C), and otolith section (D) from a Gray Triggerfish chemically marked with calcein in Octorber 2016 and died in August 2017. This fish was 2 years old.

A. Spines



B. Vertebrae

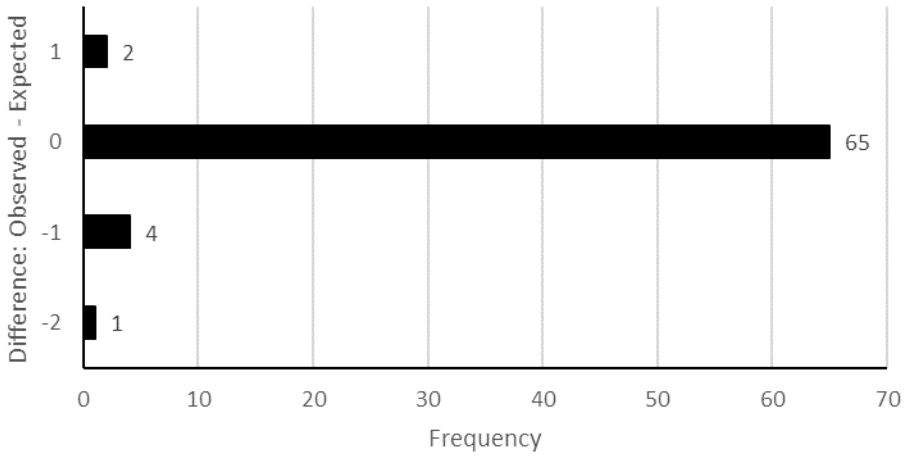
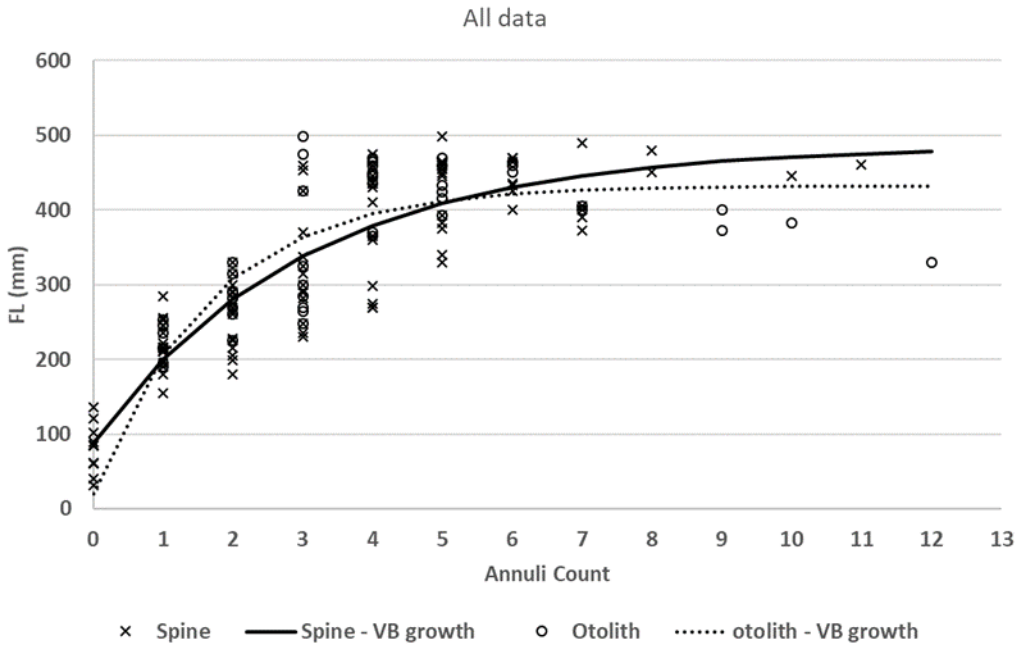


Figure 8. The difference between the observed number of growth zones after the chemical mark and the expected number of growth zones based on the marking and death dates of Gray Triggerfish in the age validation study. A. Spines and B. Vertebrae.

A.


All data
600
500
400
300
200
100
0
FL (mm)
0 1 2 3 4 5 6 7 8 9 10 11 12 13
Annuli Count
Spine
Spine - VB growth
Otolith
otolith - VB growth

B.

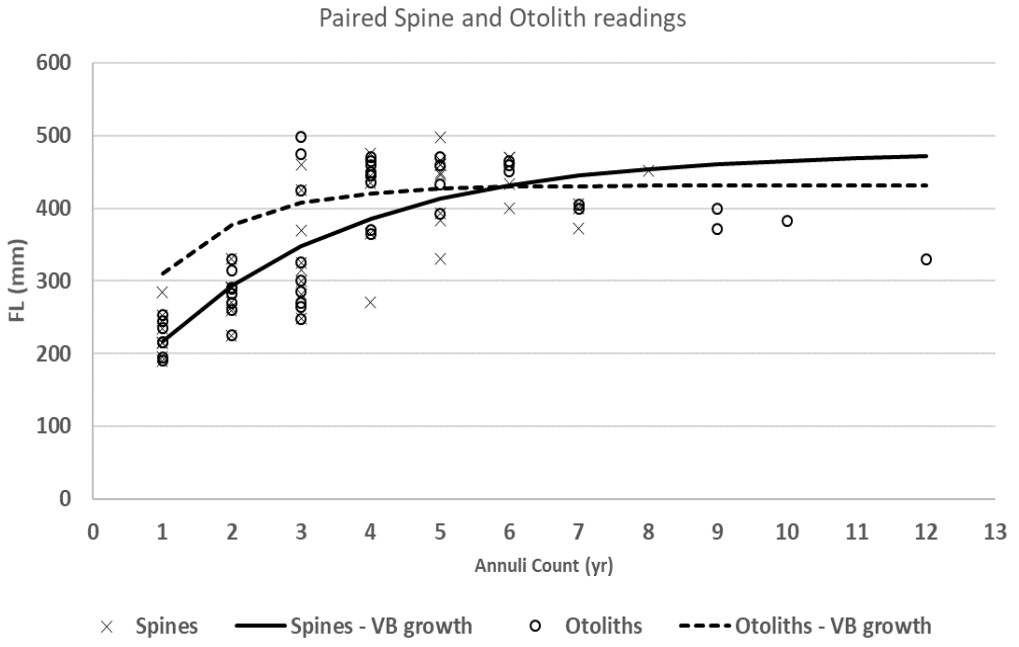

Paired Spine and Otolith readings
600
500
400
300
200
100
0
FL (mm)
0 1 2 3 4 5 6 7 8 9 10 11 12 13
Annuli Count (yr)
Spines
Spines - VB growth
Otoliths
Otoliths - VB growth

Figure 9. Von Bertalanffy growth models for Gray Triggerfish from age readings from spines and age readings from otoliths. A. all data available in the study: n = 96 for spines and n = 48 for otoliths. B. Paired age readings: n = 46.

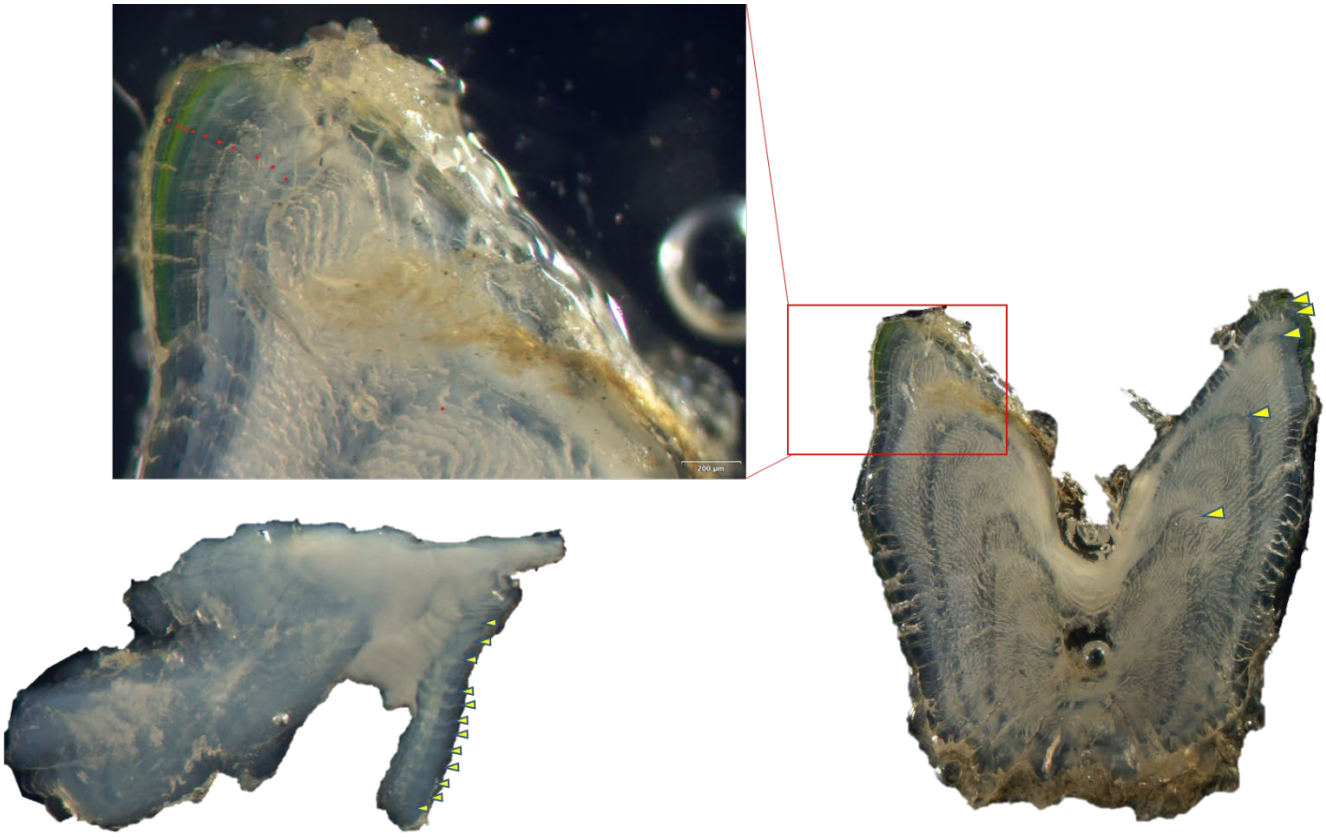


Figure 10. Gray Triggerfish otolith section and spine section indicating (yellow arrow) how initially read – age = 12 on otolith and age = 5 on spine. Inset is magnified area of spine with wide translucent zone showing the compacted growth layers (red dots). The yellow-green mark on the spine section is the calcein chemical mark.